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Isolation and identification of antifeeding compounds from vines of *Derris cavaleriei* (Leguminosae) against *Plutella xylostella* (Lepidoptera: Yponomeutidae) larvae

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Abstract: In order to determine the active compounds from *Derris cavaleriei* vines and their antifeedant activities against *Plutella xylostella* larvae, the active compounds were isolated by activity-guided fractionation with column chromatography and identified on the basis of MS and NMR data, and the antifeedant activity was determined by sandwich method. The results included that four compounds *i. e.* isolonchcarpin, magnificol, ovaliflavanone and millettocalyxin C had been isolated from the plant vines for the first time, and isolonchcarpin, magnificol and millettocalyxin C exhibited antifeeding activity, with the AFC_{50} values of 25.5, 92.8 and 115.9 mg/L against the 3rd instar larvae of *P. xylostella* at 48 h after treatment, respectively, suggesting that the three compounds are antifeedant compounds against *P. xylostella* derived from the plant vines. The elucidation of these chemicals is important not only for understanding the insect-plant relationships, but also for their potential in *P. xylostella* control.

Key words: *Derris cavaleriei*; *Plutella xylostella*; antifeedant compounds; isolation; identification; antifeedant activity; sandwich method

1 INTRODUCTION

Plutella xylostella (Lepidoptera: Plutellidae) is one of the most important insect pests on brassica crops (Liu *et al.*, 2002; Liang *et al.*, 2003; Ma *et al.*, 2005; Lü and Liu, 2007; Ling *et al.*, 2008). In general, control of *P. xylostella* was mainly dependent on chemical insecticides. Although many insecticides including some novel insecticides (*e. g.*, spinosad and indoxacarb) have been developed and introduced into the market, chemical control of *P. xylostella* is becoming less effective because many populations of this pest had developed resistance to these insecticides (Ma *et al.*, 2005; Lü and Liu, 2007). Chemical control has also led to many other problems, *e. g.*, the potential negative effects on human health and environment, disruption of natural biological control system, outbreaks of other minor insect pests and general economic considerations of agricultural production (Kranthi *et al.*, 2002; Liu *et al.*, 2002). Biological control of *P. xylostella* by natural enemies is still incredible. Thus novel control methods have been sorely needed to control the insect pests.

Previous studies indicated that secondary plant metabolites exhibiting insecticidal and especially repellent (antifeedant) activities are most acceptable and have no negative effects on human health and environment (Zabel *et al.*, 2002; Knio *et al.*, 2008), thus the exploration and utilization of botanical insecticides has been an interest in integrated pest management program (Li *et al.*, 2007; Ling *et al.*, 2008; Cheng *et al.*, 2009).

Root extracts from *Derris* spp. (Leguminosae) were reported to possess insecticidal, piscicidal and antimicrobial activities (Komalamisra *et al.*, 2005; Khan *et al.*, 2006; Li and Xu, 2007; Li *et al.*, 2007) due to interesting compounds including flavonoids, aurones, coumarins, and stilbenes. *Derris cavaleriei*, which generally grows at about 300 m altitude in Hunan and Guizhou provinces, China (Lee, 1995; Shi *et al.*, 2005), is treated as a traditional Chinese herbal medicine for killing head louse in China. Several insecticidal compounds had been isolated and identified from the methanol extracts of *D. cavaleriei* roots (Li and Xu, 2007).

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The latest results showed that the extracts from *D. cavaleriei* vines had exhibited significant antifeedant activity against *P. xylostella* larvae. However, there was no thorough investigation on antifeeding components from this plant vines. Therefore, this research is aimed at providing the basis for further exploring and utilizing the plant vines as botanical insecticides to be used in controlling of *P. xylostella*.

2 MATERIALS AND METHODS

2.1 Test plants

The vine of *D. cavaleriei* was collected in Mayang County, Hunan Province, Southern China in October 2003 and was identified by Professor LI Bin-Tao (South China Agricultural University, Guangdong Province, China). A voucher specimen was deposited at Hunan Agricultural University for further reference.

2.2 Test insects

P. xylostella colonies were collected from the container-grown cabbage and then raised on cabbage plants in cages (40 cm × 40 cm × 40 cm) under the conditions of constant temperature (24 ± 1℃), constant humidity (RH 75% ± 5%) and photoperiod of 12L: 12D in the laboratory. The 3rd instar larvae were used for antifeedant experiment.

2.3 Extraction and isolation

The air-dried and powered vines of *D. cavaleriei* (5.0 kg) were placed in a stopped conical flask and continuously extracted with methanol (50 L) for 3 d at constant room temperature (28 – 30℃) with occasional stirring, and then the solvent was filtered. After the extraction was done successively for three times, evaporation of the combined solvent under vacuum at 50℃ gave the methanolic extracts (260.0 g, 5.2% from the dried vines).

Methanolic extracts (20.0 g) suspended in the mixture (2 L) of water and methanol (4:1, V/V) were repeatedly excessively extracted in a 15 L glass-bottle with 10 L solvent of increasing polarity starting with petroleum ether, trichloromethane, ethyl acetate, and finally H_2O. The 260.0 g methanolic extracts yielded 39.0, 52.0, 78.0 and 91.0 g extracts for each above solvent, respectively.

These residues were subjected to the insecticidal assays against *P. xylostella* and the trichloromethane residues showed the most potent activity. The trichloromethane residues (52.6 g) was subjected to silica gel column chromatography (200 – 300 mesh) eluted with $CDCl_3$ and then with a gradient of $CDCl_3$-EtOAc (0 – 100%) and finally with MeOH, to give 97 fractions of 500 mL each. After verified by thin layer chromatography (TLC), fractions 35 – 43 and 67 were found to be active in the insecticidal activity evaluation, and the other inactive fractions were discarded.

Fractions 35 – 43 (148.6 mg), which were dissolved with a mixture of $CDCl_3$-EtOAc (4:1, V/V), were combined and submitted to HPLC (ODS-Symmtryprep, 5 μm, i.d. 7.8 mm × 150 mm column, MeOH-H_2O 1 : 1; 2 mL/min) to give compound A (32.6 mg), B (17.3 mg) and a mixture which was purified further by HLPC (ODS-Symmtryprep, 7 μm, i.d. 7.8 mm × 150 mm column; MeOH-H_2O 2 : 1; 2 mL/min) to give compound C (24.2 mg).

Fraction 67 (2.1 g) was processed again on silica gel column chromatography (200 – 300 Mesh) using $CDCl_3$-EtOAc (7:3) and the increasing amounts of EtOAc (0 – 100%) and finally MeOH to give 47 subfractions. Subfractions Ⅳ – Ⅶ (48.3 mg), which possessed antifeedant activity, was subjected to Sephadex LH-20 column chromatography (acetone; 3 mL each) to give compound D (47.6 mg).

2.4 Identification of compounds

^1H and ^{13}C-NMR were recorded by using a Bruker AVANCE-500 instrument. EI-MS was accomplished on a MAT-95X Pinstrument. TLC spots were visualized by UV irradiation (254 and 365 nm), by spraying with the mixture of methanol and H_2SO_4 (1:1, V/V) followed by heating.

2.5 Bioassay of antifeedant activity

The antifeedant activity of methanol extracts and compounds against the 3rd instar larvae of *P. xylostella* was determined by Sandwich method (Huang, 2000). The methanol extracts and the isolated compounds were respectively dissolved with acetone and then diluted with distilled water into five different concentrations for the bioassay. Leaf discs (15 mm diameter) of cabbage were immersed in the above five test solutions for 10 s, respectively, and then placed on the experiment table for dry. Leaf discs immersed in the solution with water and acetone were regarded as the control. The above treated leaf discs were placed in costars and 30 larvae were placed in each costar with leaf disc. Each treatment was replicated thrice. The larvae were removed when they have fed for 48 h under the laboratory conditions. The feeding area by larvae in each treatment was measured within 48 h. The antifeedant rate was calculated by the follow formula: Antifeedant rate = $100 \times (1 - T/C)\%$, where C is the feeding area by larvae in control and T is the eaten area by larvae in the treatments.

2.6 Data statistics and analysis

The median antifeedant concentration (AFC_{50}) of

compound against *P. xylostella* larvae was calculated by Probit Analysis (DPS software, version 9.5). The 95% confidence interval, values and degrees of freedom of the χ^2 goodness of fit tests, and regression equation were recorded in Table 1. Whenever the goodness of χ^2 was found to be significant ($P<0.05$), a heterogeneity correction factor was used in the calculation of the confidence limits.

3 RESULTS

3.1 Identification of the isolated compounds

Compound A: Colourless crystal. m. p. 114 – 116℃. $[\alpha]_D^{25}$ = – 125° (*c* 0.031, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ_H 5.48 (1 H, dd, *J* = 13.2, 3.0 Hz, H-2), 3.01 (1 H, dd, *J* = 13.2, 16.8 Hz, H-3a), 2.85 (1 H, dd, *J* = 3.0, 16.8 Hz, H-3b), 7.75 (1 H, d, *J* = 8.7 Hz, H-5), 6.50 (1 H, *J* = 8.7 Hz, H-6), 7.48 (2 H, m, H-2′, 6′), 7.44 (2 H, m, H-3′, 5′), 7.39 (1 H, m, H-4′), 6.65 (1 H, d, *J* = 10.0 Hz, H-4″), 5.87 (1 H, d, *J* = 10.0 Hz, H-5″), 1.47 (3 H, s, H-7″), 1.45 (3 H, s, H-8″), ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 79.83 (C-2), 44.4 (C-3), 190.5 (C-4), 128.0 (C-5), 116.0 (C-6), 159.7 (C-7), 109.5 (C-8), 157.6 (C-9), 115.6 (C-10), 139.1 (C-1′), 126.0 (C-2′, 6′), 128.8 (C-3′, 5′), 128.6 (C-4′), 111.2 (C-4″), 128.9 (C-5″), 76.8 (C-6″), 28.2 (C-7″), 28.5 (C-8″). The spectroscopic data is consistent with literature (Chen *et al.*, 1996), thus compound A is identified as isolonchcarpin, and its chemical structure is shown in Fig. 1(A).

Compound B: Colourless needle crystal. EI-MS *m/z* 424[M]$^+$. ^1H NMR (500 MHz, $CDCl_3$): δ_H: 0.76 (3 H, s, CH_3), 0.79 (3 H, s, CH_3), 0.83 (3 H, s, CH_3), 0.94 (3 H, s, CH_3), 0.96 (3 H, s, CH_3), 1.03 (3 H, s, CH_3), 1.68 (3 H, s, 7 CH_3), 4.56 (1 H, s), 4.68 (1 H, d, *J* = 2 Hz), 5.12 (1 H, t, *J* = 3.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): (δ_C 38.1 (C-1), 27.5 (C-2), 79.1 (C-3), 38.9 (C-4), 55.4 (C-5), 18.4 (C-6), 34.4 (C-7), 40.9 (C-8), 50.6 (C-9), 37.3 (C-10), 21.0 (C-11), 121.8 (C-12), 145.3 (C-13), 42.9 (C-14), 27.6 (C-15), 35.7 (C-16), 43.1 (C-17), 48.5 (C-18), 48.1 (C-19), 151.0 (C-20), 30.0 (C-21), 40.1 (C-22), 28.1 (C-23), 15.4 (C-24), 16.2 (C-25), 16.1(C-26), 14.6 (C-27), 18.1 (C-28), 109.3 (C-29), 19.4 (C-30). The spectroscopic data is consistent with literature (Siddiqui *et al.*, 1988), so compound B is identified as magnificol, and its chemical structure is shown in Fig. 1(B).

Compound C: Colourless crystal. ^1H NMR (500 MHz, $CDCl_3$): δ_H 5.44 (1H, dd, *J* = 16.8, 3.0 Hz, H-2), 2.84 (1H, dd, *J* = 16.8, 3.0 Hz, H-3a), 2.98 (1H, dd, *J* = 16.8, 13.0 Hz, H-3b), 7.61 (1 H, s, H-5), 6.12 (1 H, s, OH-7), 7.46 (2 H, m, H-2′, 6′), 7.40 (2 H, m, H-3′, 5′), 7.36 (1 H, m, H-4′), 3.42 (2 H, d, *J* = 7.2 Hz, H-1″), 5.24 (1 H, t, *J* = 7.2 Hz, H-2″), 1.78 (3 H, s, H-4″), 1.76 (3 H, s, H-5″), 3.31(2 H, d, *J* = 7.2 Hz, H-1‴), 5.30(1H, t, *J* = 7.2 Hz, H-2‴), 1.74 (6H, s, H-4‴, 5‴); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 79.6 (C-2), 44.3 (C-3), 191.2 (C-4), 125.7 (C-5, 122.0 (C-6), 159.9 (C-7), 114.8 (C-8), 159.3 (C-9), 114.6 (C-10), 139.4 (C-1′), 126.0 (C-2′, 6′), 128.7 (C-3′), 5′), 128.5 (C-4′) , 22.5 (C-1″), 121.4 (C-2″), 135.0 (C-3″), 17.9 (C-4″, 4‴), 25, 8 (C-5″, 5‴), 30.0 (C-1‴), 121.6 (C-2‴) , 134.9 (C-3‴). The spectroscopic data is consistent with literature (Nascimento and Mors, 1972), thus compound C is identified as ovaliflavanone, and its chemical structure is shown in Fig. 1(C).

Compound D: Colourless crystal. EI-MS *m/z* 322[M]$^+$. ^1H NMR (500 MHz, $CDCl_3$): δ_H 8.17 (1 H, d, J = 9.0 Hz, H-5), 7.75 (1 H, d, J = 2.0 Hz, H-5″), 7.54 (1 H, d, J = 9.0 Hz, H-6), 7.50 (1 H, d, J = 3.0 Hz, H-6′), 7.04 (1 H, dd, J = 9.0, 3.0 Hz, H-4′), 6.99 (1 H, d, J = 9.0 Hz, H-3′), 3.97 (3 H, s, CH_3O-2′), 3.95 (3 H, s, CH_3O-5′). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 159.7 (C-2), 113.2 (C-3), 178.6 (C-4), 121.8 (C-5), 109.9 (C-6), 158.3 (C-7), 117.2 (C-8), 151.1 (C-9), 118.1 (C-10), 121.4 (C-1′), 152.4 (C-2′), 113.2 (C-3′), 117.1 (C-4′), 153.6 (C-5′), 114.7 (C-6′), 104.2 (C-4″), 145.6 (C-5″), 56.2 (CH_3O-2′), 55.9 (CH_3O-5′). The spectroscopic data is consistent with literature (Sritularak *et al.*, 2002), thus the compound D is identified as millettocalyxin C, and its chemical structure is shown in Fig. 1(D).

3.2 Antifeeding activity of the isolated compounds against *P. xylostella* larvae

At the concentration of 500 mg/L, magnificol, millettocalyxin C, and isoloncharpin exhibited significant antifeedant activity, while ovaliflavanone showed no antifeeding activity to *P. xylostella* larvae.

In order to assess the antifeeding activity of the three active compounds to the larvae, AFC_{50} values of three compounds including the antifeeding compound azadirachtin A were tested. Based on the AFC_{50} values, the antifeedant potential of the three compounds to the larvae is azadirachtin A > magnificol > millettocalyxin C > isoloncharpin (Table 1).

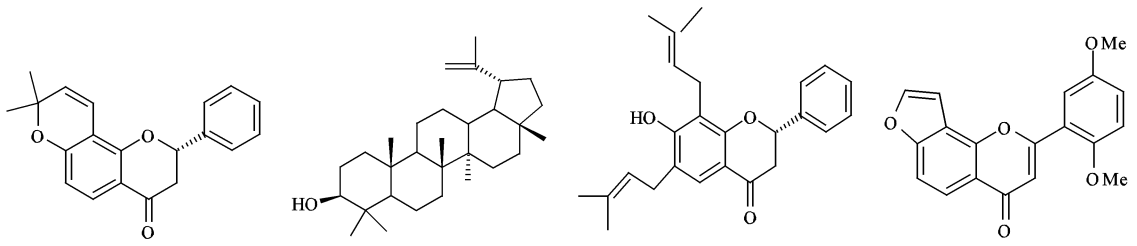

Fig. 1　Chemical structures of the compounds isolated from *Derris cavaleriei* vines

Table 1　Antifeedant activity of compounds isolated from *Derris cavaleriei* vines against the 3rd instar larvae of *Plutella xylostella* within 48 h after treatment

Compounds	Toxicity regression equation	AFC_{50} (mg/L)	95% confidence interval	χ^2
Magnificol	$y = 3.3 + 1.2x$	25.5	16.9 – 36.3	0.1067
Millettocalyxin C	$y = 2.0 + 1.5x$	92.8	61.3 – 128.7	0.0132
Isoloncharpin	$y = 2.2 + 1.2x$	115.9	73.7 – 166.5	0.2553
Azadirachtin A	$y = 3.1 + 1.8x$	12.2	8.7 – 16.3	2.2280

4 DISCUSSIONS

In this study, four compounds (magnificol, millettocalyxin C, isolonchcarpin and ovaliflavanone) were isolated and identified from *D. cavaleriei* vines for the first time. Of the four compounds, ovaliflavanone did not possess antifeeding activity at the concentration of 500 mg/L against *P. xylostella* larvae, but the other three compounds (magnificol, millettocalyxin C and isoloncharpin) possessed, with the corresponding AFC_{50} values of 25.5, 92.8 and 115.9 mg/L at 48 h after treatment, respectively. Though their AFC_{50} values are higher than that of azadirachtin A, the results still suggest that various antifeedant compounds occur in *D. cavaleriei* vines. So the crude extracts from the vines exhibited antifeeding activity to which various antifeedant compounds jointly contributed.

As is well known, flavonoids are a major group of secondary metabolites constituting of *ca.* 5% – 10% of known secondary products in plants ranging from bryophytes to angiosperms. Up to now, about 5 000 flavonoids in plants have been documented and the list is increasing steadily (Madhuri and Reddy, 1999). Flavonoids play a critical role in stress response mechanism in plants. The adaptive reaction of flavonoids in plants may play roles in defending against bacterial, fungal and viral diseases as well as insects, which is helpful to understand the importance of plant defense. As antioxidants or enzyme inhibitors, flavonoids are involved in photosynthesis and cellular energy transfer processes, and may serve as the precursor of toxic substances (Ververidis *et al.*, 2007). Both isolonchcarpin and millettocalyxin C are flavonoids, and exhibited antifeeding activity against *P. xylostella* larvae. It is supposed that the two flavonoids occurring in the vines of *D. cavaleriei* play an important role in keeping some insect pests away from the vines.

The AFC_{50} values of the three active compounds (magnificol, millettocalyxin C and isolonchcarpin) were higher than that of azadirachtin A, indicating that every compound exhibited a weak antifeeding activity. It is believed that many compounds, for example, flavonoids, terpenoids, phenolics, and alkaloids jointly or independently contribute to bioefficacy such as insecticidal, ovicidal, repellent and antifeeding activities against insect species (Isman, 2000). So many studies have focused on the determination of distribution, nature and practical use of plant extracts-derived chemical constituents with insecticidal activities (Li and Xu, 2007; Xiang *et al.*, 2009; Guo *et al.*, 2009). However, previous studies were apt to obtain and reveal the compounds that can kill the pest directly and quickly (Li and Xu, 2007), but ignored those with antifeeding and growth inhibition activities (Lee, 2000). In fact, the compounds with antifeeding or growth inhibition activity maybe universally exist in plants, and the results of this study supported the above hypothesis. Thus activity determination and elucidation of these active compounds in *D. cavaleriei* vines are helpful not only

to manage the resistant pests, but also to understand insect-plant relationships.

At present, *P. xylostella* is one of the most destructive pest of cruciferous plants throughout the world (Chen *et al.*, 2007) and is increasingly difficult to be managed because of its resistance to many insecticides (Ma *et al.*, 2005; Ling *et al.*, 2008). Phytochemical insecticides as an alternative to conventional chemical pesticides are expected to be utilized for management of this pest in cruciferous vegetable protection programs. The results in this study showed there are three flavonoids and a terpenoid with antifeeding activities in *D. cavaleriei* vines against *P. xylostella* larvae, suggesting an undeveloped resource as botanical insecticides. Thus the novel natural pesticides which are derived from antifeedant or growth inhibition compounds in plants will be expected in the future.

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湘西黑藤藤中对小菜蛾幼虫有拒食活性化合物的分离与鉴定

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摘要：为探明湘西黑藤 *Derris cavaleriei* 藤中对小菜蛾 *Plutella xylostella* 幼虫有拒食活性的化合物及其拒食活性，通过活性跟踪、利用柱色谱、借助核磁共振和质谱分离，鉴定了其拒食成分，并使用叶碟法测定各化合物的拒食活性。结果包括：首次从该植物藤中得到 4 个化合物，即 isolonchcarpin，magnificol，ovaliflavanone 和 millettocalyxin C。其中，化合物 isolonchcarpin，magnificol 和 millettocalyxin C 对 3 龄小菜蛾幼虫均有拒食活性，处理 48 h 后各化合物的拒食中浓度（AFC_{50}）分别是 25.5，92.8 和 115.9 mg/L，提示它们是该植物藤中对小菜蛾幼虫有拒食作用的化合物。阐明这些化合物不仅有助于理解植物与昆虫的关系，而且有助于评估这些化合物防治小菜蛾的潜力。

关键词：湘西黑藤；小菜蛾；拒食化合物；分离；鉴定；拒食活性；叶碟法

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